expression plasmids.

49. (New) The nucleic acid based composition of claim 23 wherein the polynucleotide that encodes the mature, mutated LF protein or an immunogenic fragment of the LF protein is a DNA molecule that does not become integrated into the genome of the mammalian subject's cells.

50. (New) The nucleic acid based composition of claim 23 wherein the polynucleotide that encodes the mature, mutated LF protein or an immunogenic fragment of the LF protein is an RNA molecule.

REMARKS

Claims 23-44 are pending in the application. Claims 25, 28-30 and 32-40 are withdrawn from consideration as being drawn to a non-elected invention. Claims 23, 24, 26, 27, 31 and 41-44 are rejected.

By the present amendment the specification is corrected to add SEQ ID NOs to sequences embedded in the text of the specification and to correct obvious typographical errors in the amino acid numbers of the sequences discussed in the text of the specification. Support for the amendments to the text of the specification is found in the Sequence Listing, page 6, lines 29-31 of the specification, and in Figures 1 and 2. The amendments, which make statements in the text consistent with the information presented in the figures of the present application, add no new matter.

By the present amendment claims 25, 28-30, 32-40, 43, and 44 are canceled without prejudice or disclaimer. By the present amendment claims 23, 24, 31, 41, and 42 are amended, and new claims 45-50 are added. Support for the amendments to claim 23 is found in original claim 31; page 3, lines 12-18; page 9, lines 19-23; page 13, lines 2-9; page 14, lines 14-16; and page 16, lines 5-7. Support for the amendment to claim 24 is found on page 7, lines 1-18. Support for the amendment to claim 31 is found on page 5, lines 18-20. Support for the amendment to claim 41 and new claims 45-48 is found on page 11, lines 10-23, and page 13, lines 2-3. Support for new claims 49 and 50 is found on page 10, line 13-18. The amendments and new claims add no new matter. A document entitled "VERSION WITH MARKINGS TO

SHOW CHANGES MADE" showing the additions as underlined and the deletions in brackets is attached hereto.

In view of the above-described amendments and following remarks, reconsideration of claims 23, 24, 26, 27, 31, and 41, and consideration of new claims 45-50 is respectfully requested.

Drawings

The drawings were objected to by the Draftsperson. Enclosed herewith are Figures 1 and 2 corrected in accordance with suggestions from the Draftsperson.

Abstract

Two abstracts have previously been submitted. One abstract was submitted as page 26 of the original disclosure and another abstract was submitted on April 26, 2001 as paper #6. Applicants hereby cancel the abstract submitted with the original disclosure on December 21, 2000.

Specification

In accordance with 37 CFR 1.821 (d), the application has been amended to identify sequences in the specification with sequence identifiers. Sequence identifiers have been added to two sequences on page 13 and two sequences on page 15 of the application. These four sequences are already present in the filed sequence listing.

Claim Rejections - 35 USC § 112

The Patent Office has rejected claims 23, 24, 26-27, 31, 41, 42 and 44 under 35 U.S.C 112, first paragraph. The Patent Office stated

> [T]he specification, while being enabling for a nucleic acid based immunogenic composition, does not enable a vaccine and immunogenic fragments of such a composition. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected to make and/or use the invention commensurate in scope with these claims.

Claim 44 has been cancelled, rendering the rejection of this claim moot.

Claim 23 as amended recites that the nucleic acid based composition protects mammalian

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subjects against challenge with lethal toxin of *B. anthracis*. Claim 23, as amended, further recites that the composition comprises a polynucleotide that encodes a full-length, mature, mutated LF protein that lacks metalloproteinase activity or an immunogenic fragment of the *B. anthracis* LF protein. Claim 23 further recites that the immunogenic fragment of the LF protein comprises amino acid amino acid 43 through amino acid 285 of SEQ ID NO. 2

Claim 24 as amended recites that the nucleic acid based composition further comprises a polynucleotide that encodes a full-length, mature PA protein or an immunogenic fragment thereof. Claim 24, as amended, further recites that the immunogenic fragment of the PA protein comprises comprises amino acid 204 through amino acid 764 of SEQ ID NO. 4

Applicants have described how to make the compositions recited in amended claims 23 and 24 of the present application. (See pages 10, 11, 13 and 15 of the specification.) Applicants have also provided evidence that compositions that comprise the LF fragment recited in claim 23 as amended is capable of protecting a mammalian subject against challenge with lethal anthrax toxin. (See Examples 1 and 2) In view of the fact that this LF fragment lacks metalloproteinase activity, there is no reason to doubt that a polynucleotide which encodes a full-length, mature, mutated LF protein that has been mutated to eliminate its metalloproteinase activity would be incapable of producing a similar result. Furthermore, applicants have shown that protection against lethal anthrax toxin and lethal anthrax spores is increased when mammalian animals are injected with DNA plasmids comprising both a polynucleotide that encodes a fragment of the LF protein and polynucleotide that encodes a fragment of a PA protein, as recited in claim 24 as amended (See Examples 1 and 2.) Accordingly, it would not require undue experimentation for one of ordinary skill in the art to make and use the immunogenic compositions recited in amended claims 23 and 24.

The Patent Office further stated:

> The specification fails to set forth sufficient evidence showing that the claimed vaccine complex could be made with dual molecules of claim 42 wherein the first and second polynucleotides are incorporated in the same viral vector.

Claim 42 has been amended to recite that polynucleotides encoding the LF and PA proteins "are incorporated into the same or separate mammalian expression vectors." The claim,

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expression plasmids encoding an immunogenic fragment of LF and an immunogenic fragment of PA can be used to achieve the desired results. (See page 16, lines 22-23 of the specification.) On the basis of the information provided in the specification, it would require nothing more than routine experimentation to determine the ratios of the two polynucleotides recited in claim 24 that induce protection against challenge with anthrax lethal toxin or anthrax spores in a different mammalian subject.

With respect to the terms "first polynucleotide" and "second polynucleotide", these terms were included in the claims in order to distinguish the polynucleotide that encodes a full-length, mature, mutated LF protein or an immunogenic fragment thereof (i.e., the first polynucleotide) from the polynucleotide that encodes a full-length, mature PA protein or an immunogenic fragment thereof (i.e., the second polynucleotide). Applicants included the terms "first" and "second" in the claims to ensure that the reader would not mix up the two polynucleotides that are included in the immunogenic composition of claim 24, particularly in the claims that depend therefrom. However, in view of the Examiner's comments, applicants have removed these terms from the claims 23, 24, 31, and 42, and added language that characterizes the two polynucleotides into each of these claims.

The Patent Office further stated.

> Two types of anthrax vaccine are licensed for use in humans.....The use of live attenuated STI-1 occasionally results in general and local adverse responses. Furthermore it was reported that STI-1 vaccine has a relative low immunogenicity....While cell-free PA based vaccines appear to be safer; they require numerous boosters...It appears, therefore, there is a need for a safe and more efficient vaccine, which could generate stable and prolonged immunity in humans.

Applicants agree that a vaccine that is more safe, efficient, and that generates more stable and prolonged immunity in humans than existing vaccines is desirable. However, the enablement requirement of 35 USC §112 does not require that Applicant's present immunogenic composition be an improvement over existing vaccines for anthrax. To be patentable, applicants invention need not be of greater usefulness than the prior art (In re Holmes 63 F.2d 642). Thus, it is not necessary for applicant's composition to require only one injection, or generate a more stable and prolonged immunity than existing anthrax vaccine in order to meet the enablement requirement of §112.

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therefore, now encompasses viral expression vectors as well as other vectors, such as eukaryotic expression plasmids.

It is well known in the art that many different types of vectors can be made to encode and express greater than one gene. With regard to viruses, for example, U.S. Patent No. 6,140,087 by Graham, et al. discloses adenovirus vectors that encode two or more genes, as follows:

> Because of the large capacity of the vectors provided herein, multiple inserts of foreign genes can be placed in the E1 cloning site. For example, two or more genes encoding different antigens, or genes encoding useful proteins, can be combined with genes encoding chemically selectable markers. (Column 3, lines 34-40; emphasis added).

Plasmid vectors for expression of two genes are also known in the art (e.g., see the pVIVO-mcs plasmids from InvivoGen; http://www.invivogen.com/index.htm). Thus, there is no reason to doubt that polynucleotides encoding a full-length, mature, mutated LF protein (or an immunogenic fragment thereof) and a full-length, mature PA protein (or an immunogenic fragment thereof) could not be incorporated into and expressed from the same mammalian expression vector as well as from separate mammalian expression vectors, as recited in claim 42, as amended.

The Patent Office further stated:

> Further, the specification does not allow one of skill in the art to fully understand the association between the multiple components present in the "complex". For example, the optimal amounts or proportions of different "bacterial protein fractions", i.e., protective antigen protein and/or lethal factor protein, that should be present in the complex such that the complex can accomplish its alleged immunogenic and/or preventive functions are not disclosed. It is also not clear from the specification what is encompassed by the first polynucleotide and the second polynucleotide. What sequences encompass each of these polynucleotides?

Respectfully, Applicants are not required to teach optimal amounts in order to meet the enablement requirement of 35 USC §112. Applicants are not even required to teach all of the ratios that can be used. All that is required is the Applicants provide sufficient guidance so that one of ordinary skill in the art can make and use the claimed compositions without undue experimentation.

Applicants have provided examples showing that a one to one ratio of eukaryotic

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The Patent Office also states:

> In the instant specification no art recognized *in vitro* or *in vivo* models are shown in which protection is produced from instantly claimed invention and correlated to protection in humans.

Applicants have conducted studies in the mouse model system, an *in vivo* model that has been used by others to test the ability of compositions to induce protection against anthrax (See paragraph 2 of the Rule 1.132 of Dr. Darrel Galloway, which is attached hereto) Applicants have provided working examples (see Examples 2 and 3) which clearly show that the nucleic acid compositions recited in claims 23 and 24, as amended, stimulate production of antibodies (see Table 2, p. 18), are protective against lethal anthrax toxin challenge (see Table 1, p. 17 of specification), and are protective against lethal anthrax spore challenge (see Table 3, p. 19 of specification) in this animal model. The Patent Office has provided no scientific reason as to why the mouse model is not suitable for testing a composition's ability to induce protection against the toxin that is produced by *B. anthracis* in other mammals, including humans. Moreover, Applicants have conducted further studies with a second animal model, namely rabbits, and achieved the same results, when such animals were injected with polynucleotides that encode the same LF and PA protein fragments, as well as with polynucleotides that encode s full-length, mature, mutated LF protein and a full-length, mature PA protein. (See paragraph 2 of the Rule 1.132 Declaration of Dr. Galloway) Applicants are not required to test the compositions recited in claims 23 and 24 in humans in order to meet the enablement requirement of §112.

In view of the above-described amendments and remarks, Applicants submit that claims 23 and 24, as amended, and the claims that depend therefrom are all fully-enabled.

Claim Rejections - 35 USC § 102

The Patent Office has rejected claims 23, 24, 26, 27, 31 and 41-44 under 35 U.S.C. 102 (b) as being anticipated by Leppla et al., US Patent No. 5,591,631 (hereinafter, "Leppla

Leppla does not disclose a polynucleotide which encodes a full-length, mature mutated LF protein or an immunogenic fragment of LF protein, and which is operably linked to a promoter that drives expression of the full-length, mature mutated LF protein or the immunogenic fragment of LF protein in cells of a mammalian subject, as recited in claim 23, as

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amended. In addition, Leppla does not disclose a polynucleotide which encodes a full-length, mature *B. anthracis* PA protein or an immunogenic fragment thereof, and which is operably linked to a promoter that drives expression of the full-length, mature PA protein or the immunogenic fragment thereof in cells of a mammalian subject, as recited in claim 24, as amended. Furthermore Leppla does not disclose incorporation of these polynucleotides into a mammalian expression vector as recited in claim 42, as amended.

The prokaryotic promoters that are used by Leppla are different functionally and in their sequence from the eukaryotic promoters recited in amended claims 23 and 24 of the above-described application. (See paragraph 3 of the Rule 1.132 Declaration of Dr. Galloway, which is attached hereto.) Similarly, the prokaryotic expression vectors that are used by Leppela to produce his fusion proteins are different from the mammalian (eukaryotic) expression vectors that are recited in the claims of the instant application. (Id.) The nucleic acid compositions of Leppla are specifically designed to express the Leppla fusion proteins in prokaryotic hosts, while the nucleic acid compositions of the instant application are designed to express LF protein or fragments thereof and PA proteins or fragments thereof in the mammalian (eukaryotic) hosts that are to be immunized.

Leppla discloses prokaryotic transcriptional promoters such as the lactose, tryptophan, and beta-lactamase promoter systems as well as phage lambda promoters. (See column 6 , lines 50-56 of Leppla.) These are promoters that have been derived from prokaryotic bacteria and bacterial phages. (Id.) Bacterial promoters do not initiate transcription of nucleic acids in mammalian (eukaryotic) cells.(Id.) Bacterial (prokaryotic) promoters do not drive expression of the protein encoded by the polynucleotides to which such promoters are linked in mammalian (eukaryotic) cells. (Id.)

In contrast, claims 23 and 24 of the instant application specifically recite eukaryotic promoters that drive expression of the LF protein (or an immunogenic fragment thereof) and the PA protein (or an immunogenic fragment thereof) in mammalian (eukaryotic) cells. Furthermore, the instant specification describes mammalian (eukaryotic) promoters such as the human cytomegalovirus (CMV) immediate-early enhancer promoter. (See page 9, lines 29-31 of the instant application.) Mammalian (eukaryotic) promoters, such as CMV, do not function in prokaryotic, i.e., bacterial systems. (Id.)

Leppla also indicates that the hosts for his expression vectors are microbial hosts or

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prokaryotic hosts, and that the prokaryotic expression vectors disclosed therein "will contain expression control sequences compatible to the host cell."(See column 6, lines 44-53 of Leppla.) In contrast, the eukaryotic control sequences disclosed in the plasmids of the present application contain viral and eukaryotic expression control systems that are compatible with eukaryotic or mammalian host cells. In addition to a viral promoter which is used to express recombinant protein in eukaryotic or mammalian cells, the plasmid shown in Figure 3 of the instant application comprises additional mammalian transcriptional regulatory sequences, namely the CMV intron A and SV40 late poly(A) sequences. (Id.) These sequences facilitate mRNA processing and, thus, enhance gene expression. Such sequences play no role in prokaryotic gene expression and are not part of the nucleic acid compositions of Leppla. (Id.) Therefore, in a variety of ways, the nucleic acid compositions of Leppla are different from the nucleic acid compositions described in the instant application.

Also, contrary to the examiner's statement on page 9 of the office action, Leppla does not teach, or even mention viral vectors. Although HIV is mentioned in Leppla (column 8, lines 4-10), killing of HIV infected cells is being described. There is no disclosure in Leppla of HIV, or any other virus, being used as a vector for gene transfer or expression.

Lacking a disclosure of a promoter that drives expression of a full-length, mature, mutated LF protein or a fragment thereof in mammalian cells, Leppla does not anticipate claim 23 as amended, or the claims that depend therefrom. Lacking a disclosure of a promoter that drives expression of a full-length, mature PA protein or an immunogenic fragment thereof in mammalian cells, Leppla does not anticipate claim 24, as amended, or the claims that depend thereofrom. Lacking a disclosure of incorporation of a polynucleotide that encodes a full-length, mature, mutated LF protein or a fragment thereof and a polynucleotide that encodes a full-length, mature PA protein or an immunogenic fragment thereof into mammalian expression vectors, as recited in claim 41 as amended, Leppla does not anticipate claim 41 or the claims that depend therefrom.

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In view of the above-described amendments and remarks, it is submitted that claims 23, 24, 26, 27, 31, 41, and 42 and new claims 45-50 are now in condition for allowance. Prompt notice of such allowance is respectfully requested.

Respectfully submitted,

Date: December 12, 2002

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VERSION WITH MARKINGS SHOWING CHANGES MADE

IN THE SPECIFICATION:

Page 3, paragraph beginning on line 12

The present invention provides methods of inducing an immune response which protects an animal subject from lethal infection with *Bacillus anthracis* (*B. anthracis*). One method comprises administering an effective amount of wild-type, or preferably a mutated form of [,] *B. anthracis* lethal factor (LF), or an immunogenic fragment thereof to the subject. In one embodiment the mature, wild-type LF protein comprises the amino acid sequence[, SEQ ID NO.2] shown in Figure 1B, i.e., amino acid 34 through amino acid 809 of SEQ ID NO. 2. In one embodiment the LF fragment comprises amino acid 9 through amino acid 252 of the sequence[, SEQ ID NO:2,] shown in Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2. A second method comprises administering an effective amount of a mutated LF protein or a fragment thereof and an effective amount of the *B anthracis* protective antigen (PA) or an immunogic fragment of the PA protein to the subject. In one embodiment, the immunogenic fragment of the *B anthracis* protective antigen comprises consecutively amino acid 175 through amino acid 735 of the amino acid sequence[, SEQ. ID NO: 4,] shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4. A third method comprises administering a polynucleotide or nucleic acid comprising a sequence encoding *B. anthracis* LF protein or a fragment thereof to the subject. In one embodiment the polynucleotide which encodes the full-length mature LF protein comprises consecutively nucleotide 100 through nucleotide 2430 of the sequence, SEQ ID NO. 1, shown in Figure 1A. In one embodiment the polynucleotide which encodes an LF fragment comprises consecutively nucleotide [125]124 through nucleotide 855 of the sequence, SEQ ID NO:1, shown in Figure 1A. A fourth method comprises administering a polynucleotide which comprises a coding sequence for a mutated LF protein or immunogenic fragment thereof and a polynucleotide which comprises a coding sequence for the *B. anthracis* PA protein or an immunogenic fragment thereof to the subject. In one embodiment, the nucleotide sequence encoding the full-length, mature PA protein comprises consecutively nucleotide 88 through nucleotide 2295 of the sequence, SEQ. ID NO: 3, shown in Figure 2A. In one embodiment, the nucleotide sequence which encodes an immunogenic fragment of the PA protein, comprises consecutively nucleotide 610 through nucleotide 2295 of the sequence, SEQ

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ID NO: 3, shown in Figure 2A. The present methods stimulate or increase the level of antibodies which inactivate the *B. anthracis* lethal toxin in the subject.

Page 4, paragraph beginning on line 17

Figure 1A shows a nucleotide sequence, SEQ ID NO:1, of a DNA which encodes wild-type *B. anthracis* LF protein; [and] Figure 1 B shows the amino acid sequence[, SEQ ID NO. 2, derived therefrom] of the full-length mature, wild-type LF protein, i.e. amino acids 34 through amino acid 809 of SEQ ID NO. 2; Figure 1 C shows the amino acid sequence of the polypeptide encoded by eukaryotic encoded by the eukaryotic expression plasmid pCLF4.

Page 4, paragraph beginning on line 19

Figure 2A shows a nucleotide sequence, SEQ ID NO.3, of a DNA which encodes a wild-type *B. anthracis* PA; [and] Figure 2B shows the amino acid sequence[, SEQ ID NO.4,] of the full-length, mature wild-type PA protein [derived therefrom], i.e. amino acid 30 through amino acid 764 of SEQ ID NO. 4; and Figure 2C shows the amino acid sequence of the polypeptide encoded by the eukaryotic expression plasmid pCPA.

Page 6, paragraph beginning on line 23

In one embodiment the LF protein immunogenic fragment comprises amino acid 9 through amino acid 252 of the amino acid sequence[, SEQ ID NO: 2,] shown if Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2. The term LF protein fragment, as used herein, also encompasses LF protein fragments whose sequence differs from the sequence shown in Figure 1C. Such polypeptides have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "LF protein fragment reference sequence", which begins with amino acid 9 and extends through amino acid 252 of the sequence shown in Figure 1B. Such variants, when injected into an animal, elicit production of antibodies that bind to the mature wild-type LF protein, i.e., the LF protein whose sequence is depicted in Figure 1B.

Page 7, paragraph beginning on line 1

In another aspect, the peptide-based immunogenic composition comprises a mutated LF

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protein or immunogenic fragment of LF protein and the *B. anthracis* PA protein or an immunogenic fragment thereof. The full-length, wild-type PA protein has a molecular weight of 83 kDA and comprises 735 amino acids. In one embodiment, the full-length, wild-type, mature PA protein comprises the amino acid sequence[, SEQ ID NO: 4,] shown if Figure 2B, i.e. amino acid 30 through amino acid 764 of SEQ ID NO. 4. The term PA protein, as used herein also encompasses wild-type and mutated PA proteins whose sequence differs slightly from the sequence shown in Figure 2B. Such variants have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "PA protein reference sequence" shown in Figure 2B. Suitable variants elicit production of antibodies that bind to the wild-type PA protein, i.e., the PA protein whose sequence is shown in Figure 2B.

Page 7, paragraph beginning on line 11

In one embodiment the PA protein fragment comprises amino acid 175 through amino acid 735 of the amino acid sequence[, SEQ ID NO: 4,] shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4. The term PA protein fragment, as used herein, also encompasses proteins whose sequence differs slightly from the sequence shown in Figure [1]2C. Such variants have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "PA protein fragment reference sequence", which begins with amino acid 175 and extends through amino acid 735 of the sequence shown in Figure 2B. Suitable variants of the PA fragment elicit production of antibodies that bind to the wild-type PA protein, i.e. the PA protein whose sequence is shown in Figure 2B .

Page 13, paragraph beginning on line2

The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the LF protein. The plasmid construct pCLF4 encodes the LF protein fragment consisting of amino acids 9-252 which includes the PA binding site. This plasmid was constructed from a PCR-amplified fragment using the primers 5'-CTGAAACCATCACGTAAAA-3' SEQ ID NO.5 and 3'-AGCAAGAAATAAATCTATAGTCTAGA-5' SEQ ID NO.6 which contain *Xba* cut sites. The

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Xba-digested PCR and pCI plasmid fragments were ligated to form the pCLF4 plasmid used in these studies. The resulting plasmid construct pCLF4 does not contain a signal sequence for secretion of the expressed gene product. All plasmids were purified from *E. coli* DH5α using the Endo-free plasmid preparation kits (Qiagen) and resuspended in PBS before use.

Page 15, paragraph beginning on line 17

The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the LF protein. The gene fragment encoding amino acids 175-735 of the PA protein was PCR amplified using the plus strand primer (5'-CTCGAGACCATGGTT-3'; SEQ ID NO.7) and minus strand primer (3'-TAAGGTAATTCTAGA-5'; SEQ ID NO.8) using pYS2 as a template (Welkos 1988; Singh 1994). Included in the primer sequences are *Xho* and *Xba* restriction cut sites, respectively. The PA gene fragment expressed in these studies represents the PA_{63} protease-cleaved fragment of the full-length 83 kDa protein that is active in vivo (Gordon 1995). The PCR reaction product was digested with *Xho*I and *Xba* and ligated into the pCI vector which had been cut with the same two restriction enzymes.

IN THE CLAIMS:

23. (Three Times Amended) A nucleic [-]acid based immunogenic composition for [preparing a vaccine which protects] protecting a mammalian subject against challenge with lethal toxin [infection with] of *B. anthracis*, said immunogenic composition comprising a [first] polynucleotide which encodes a full-length, mature mutated lethal factor (LF) protein or an immunogenic fragment of [said mutated]LF protein, said [first] polynucleotide being operably linked to a promoter which drives expression of the full-length, mature mutated LF protein or the immunogenic fragment of [said mutated] LF protein in cells of the mammalian subject; [and a pharmaceutically acceptable carrier or diluent] wherein the full-length, mature mutated LF protein comprises a sequence which is the same as the sequence of the full-length, mature wild-type LF protein except for a mutation that eliminates the metalloproteinase activity of the full-length, mature, mutated LF protein, and wherein the immunogenic fragment of LF protein comprises amino acid 42 through amino acid 285 of SEQ ID NO. 2.

24. (Twice Amended) The nucleic [-] acid based immunogenic composition of claim 23, wherein the nucleic acid based composition further [comprising a second isolated] comprises a polynucleotide which encodes a full-length, mature *B. anthracis* protective antigen (PA) protein or an immunogenic fragment thereof to the subject, wherein said [second] polynucleotide which encodes a full-length mature *B. anthracis* PA protein or an immunogenic fragment thereof is [being] operably linked to a promoter which drives expression of the full-length, mature PA protein or the immunogenic fragment thereof in cells of the mammalian subject, wherein said full-length mature *B. anthracis* PA protein comprises amino acid 30 through amino acid 764 of SEQ ID NO. 4, and wherein said immunogenic fragment of the *B. anthracis* PA protein comprises amino acid 204 through amino acid 764 of SEQ ID NO. 4.

31. (Twice Amended) The nucleic [-] acid based immunogenic composition of claim 23 wherein the [first] polynucleotide encodes [an immunogenic fragment of said mutated LF protein, said] a polypeptide comprising [a sequence which is at least 90% identical to a sequence extending from amino acid 9 through amino acid 252] sequentially amino acid 34 through amino acid 719 of the amino acid sequence set forth in SEQ ID NO. 2, an amino acid other than glutamic acid, and amino acid 721 through amino acid 809 of the sequence set forth in SEQ ID NO. 2.

41. (Once Amended) The nucleic [-] acid based immunogenic composition of claim 23 wherein the [first] polynucleotide is incorporated into a mammalian expression [viral] vector.

42. (Once Amended) The nucleic-acid based immunogenic composition of claim 24 wherein the [first] polynucleotide that encodes the full-length, mature mutated LF protein or immunogenic fragment thereof and the [second] polynucleotide that encodes the full-length, mature PA protein or immunogenic fragment thereof are incorporated into the same or separate [viral] mammalian expression vectors

ABSTRACT

Methods of inducing an immune response which protects a susceptible animal subject from lethal infection with *Bacillus anthracis* (*B. anthracis*) are provided. One method comprises administering *B. anthracis* lethal factor (LF) or an immunogenic fragment thereof to the subject. A second method comprises administering LFor an immunogenic fragment thereof and the *B anthracis* protective antigen (PA) to the subject. A third method comprises administering a polynucleotide which encodes *B. anthracis* LF or an immunogenic fragment thereof to the subject. A fourth method comprises administering a polynucleotide which encodes LF or an immunogenic fragment thereof and a polynucleotide which encodes the *B. anthracis* PA to the subject. The present invention also relates to a protein or peptide based-immunogenic composition for preparing a vaccine which is capable of prophylactically protecting a subject against lethal effects of infection with *B. anthracis*.

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